MOLECULAR DYNAMICS SIMULATION OF MIXED LIPID BILAYER WITH DPPC AND MPPC: EFFECT OF CONFIGURATIONS IN GEL-PHASE

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LIST OF SYMBOLS AND ABBREVIATIONS

DPPC	1, 2-Dipalmitoyl-s n-Glycero-3-Phosphocholine
MPPC	1-Palmitoyl-2-Hydoxy-sn-Glycero-3-Phosphocholine

SUMMARY

Liposomes, spherical nanoparticles composed of phospholipid bilayers, have been suggested as a drug delivery system for potent chemotherapeutics. As the lipid structure transitions from gel to liquid-crystalline the structure reaches its maximum permeability, which then the encapsulated drugs can be released. As this phase transition occurs with changes in temperature, it has been suggested to incorporate lysolipid as part of the phospholipid bilayer systems to control this transition temperature. This study runs fully atomistic molecular dynamics (MD) simulations with flat liposome mixed-dispersed and mixed-island model. Mixed-dispersed model has lysolipid evenly distributed across the structure; Mixed-island model has lysolipid aggregated in the middle of the structure. The system is comprised of 10 percent lysolipid and 90 percent Dipalmitoyl Phosphatidyl Choline (DPPC). MonoPalmitoyl Phoshatidyl Choline (MPPC) is used as lysolipid. The changes in structure through the phase transition are investigated.

CHAPTER 1

INTRODUCTION

Lipid bilayer is one of essential parts of the living organisms that draws a boundary between in and out of the cell [1]. Drug encapsulations within vesicles are thought of as one of the most promising mechanisms of drug delivery. More specifically thermos-sensitive vesicles that will transition from gel to liquid crystalline phase results in peak permeability, when all of the contents are released [2].

Needham proposed lysolipid (MPPC) incorporated mixed with DPPC lipid bilayer system for the lipid to have a specific phase transition temperature [3]. There have been many experiments that tested various mixed composition of lysolipid (or other substances like cholesterol) with the lipid to design a most effective system [4-6]. There have been simulations at molecular and coarse grain models to see how the mixed lipid system transitions from gel to liquid crystalline phase.

There are studies that focus on the percent compositions of lysolipid in mixed lipid bilayer systems [2-4, 6]. Yet, there is a lack of understanding in how the lysolipid is distributed within the lipids in mixed lipid bilayer system. The idea of different lipid distributions was suggested for dimyristoylphosphatidylcholine (DMPC) and DPPC [7], yet such idea for DPPC and lysolipid mixed systems are yet to be discussed.
As our group believes that the distribution of lysolipid within the structure can play a very important role, we propose two different models of lysolipid distributions. We have a ‘dispersed’ structure where lysolipid structures are uniformly distributed within other lipid molecules. We have an island structure where lysolipid structures are aggregated like an island in the sea of lipids. We will observe phase transition of mixed system at two different lay out of the distributions.

This study will help understand how mixed lipid bilayer systems distribute themselves, providing a valuable insight on an important property of the mixed lipid

bilayer system. Further, knowing how the lysolipid are positioned in relation to the DPPC lipid bilayers will eventually help design a better drug delivery system.

CHAPTER 2

LITERATURE REVIEW

Lipid systems have a transition temperature, specific to their compositions. During the phase transition, the permeability of the bilayer goes through a dramatic increase, which will result in release of the drug encapsulated within the lipid bilayer. A pure DPPC lipid bilayer has a transition temperature that is much higher than the normal body temperature. To make a drug delivery system that could be activated upon mild hyperthermia, Needham proposed the mixed liposome structure that is composed of 2 to 30 mole percentages of lysolipid mixed with phospholipids [3]. These mixed lipid structures were specifically designed for the drug delivery. The liposome will release drugs as it transitions from gel to liquid crystalline phase as they are exposed to an environment of higher temperature. The significance of incorporating lysolipid to the phospholipids stands on the lower transition temperature. The mixed lipid systems overcome the previous challenge in application of liposome as a drug delivery system due to its high transition temperature. Thus, this lower transition temperature—closer to temperature ranges of hyperthermia (artificial heating of the local site) or natural causes like infection—makes drug delivery system via liposome a much more realistic model for a clinical use [3].

There have been experiments and simulations that looked at various mixed compositions of lysolipid (or cholesterol) as an integrated part of the phospholipids system. For example, a study of a coarse-grained molecular dynamics on permeability of membranes in mixed lipid system composed of 1, 2-Dipalmitoyl-s n-Glycero-3-Phosphocholine (DPPC) and 1-Palmitoyl-2-Hydoxy-sn-Glycero-3-Phosphocholine

(MPPC) (identical to the structure Needham [3] had proposed) has shown that small percentages (15% or less) of MPPC is best for the stabilization of the gel phase [4]. The continued experiment and simulations on the mixed lipid systems have shown rapid release of arsenic trioxide at 5 and 10 percent of lysolipid compositions [6]. The studies that have investigated phospholipids system with incorporation of cholesterol [8] deserve some attention too as MPPC exhibits some characteristics that are similar to that of cholesterol [3]. Similar to the mixed lipid structures composed of DPPC and MPPC, DPPC and cholesterol, mixed bilayer molecule dynamics also show high dependence on temperature and cholesterol percent compositions [2, 8].

All of the reports stated above focus on the mixed liposome system with varying lysolipid compositions transitions from lower to higher temperature. Yet, the distribution of the lysolipid within the mixed liposome system is not considered. In one study, they have proposed three different composition distributions: minima, random, and evenly [7]. However, the system of liposomes were composed of two different types of phospholipids composed of DPPC and dimyristoylphosphatidylcholine (DMPC), and such study of distribution of lysolipid in a mixed bilayer system has not yet been done (according to our research). Our study focuses on how the distribution of the lysolipid affects the transition from gel to liquid crystalline phase at 10 percent composition, which is known to have shown significant permeability with distinctive transition characteristics.

CHAPTER 3

MATERIALS AND METHODS

DPPC, MPPC, and water molecules were sketched using 3D sketcher menu within cerius2 in nano. Charges were assigned via Maestro in nano2. Using DPPC and MPPC molecules, the lipid bilayer was built. Same percent composition of DPPC (90%) and MPPC (10%) molecules were used for both dispersed and island model. Total of 120 lipids were used. Energy minimization was initially performed briefly in cerius2. Using DREIDING force field with LAMMPS in logos, the initial lipid systems of structures (without water) were heated from 15.0 K to 80.0 K in 50 ps. 80 K to 300 K in 200 ps. NPT ensemble used. The lipid system was simulated in NVT for short durations while getting the system to reach equilibrium. Water molecules went through the same processes of energy minimization with LAMMPS and DREIDING force field. Total of 6920 water molecules were added to the both of lipid structures. The entire system including water molecules was prepared by going through identical energy minimization procedures listed above. Simulation was performed using DREIDING Force Field in LAMMPS, under NPT ensemble for 6ns at each temperature. Using the last 500ps of the results, following calculations were performed.

Interface formation energy, radial distribution function, and density profiles. In order to calculate interface formation energy, the following equation is used:

$$IFE = \frac{E_{total} - (nxE_{single\ lipid} + E_{water})}{n}$$

E_{total} is the overall energy of the entire system. n is number of lipids in the entire system. $E_{single\ lipid}$ is the overall energy of a single lipid. E_{water} is the overall energy of water molecules within the system.

Molecule Preparation

- Cerius2, Accelrys and Jaguar, Schrödinger

	DPPC	MPPC	Water
Number of Molecules	108	12	6920

- Quantum mechanical Density Functional Theory (DFT) for charge calculation

Software	Force Field	Boundary Condition
LAMMPS	Dreiding	Periodic

System Construction

- Cerius2, Accelrys | Energy minimization

Molecular Dynamics Simulation Conditions

Basis Set	Functional	Analysis
6-31G**	B3LYP	Mulliken Population

Solvent	Ensemble	Pressure
Water	NPT	1 atm

Cell Parameters

	300 K		305 K	
	Dispersed	Island	Dispersed	Island
Lx (Å)	67.86	62.09	68.21	61.53
Ly (Å)	50.22	54.39	49.52	55.13
Lz (Å)	102.76	103.76	103.78	103.51

Table 1: Simulation Details

RDF is a useful calculation to show structure of the system, especially in liquid phase. Pair correlation function represents the probability of finding B atoms at a distance r from A atoms, averaged over the equilibrium trajectory as shown.

$$g_{A-B}(r) = \left(\frac{n_B}{4\pi^2 \Delta r}\right) / \left(\frac{N_B}{V}\right)$$

n_B is the number of atoms B located at the distance r in a shell of thickness Δr from atom A. N_B is the number of B particles in the system. V is the total volume

Density of water, DPPC, MPPC, and the total system were calculated using cerius2 across the z-axis of the simulation box. The results on the right and the left were averaged as the molecules in the simulation box are positioned to mirror each other.

CHAPTER 4

RESULTS AND DISCUSSIONS

Simulations looked at how variations in distribution of lysolipids in mixed lipid system affect their properties at 305K to examine which configuration—dispersed or island—is more favorable. Interface formation energy, radial distribution function, density profile, and area per lipid were calculated for each configurations.

Interface formation energy

Results are presented in Table 2. Total energy had some differences with increase in temperature. When interface formation energy was calculated, the island structures had slightly higher energy compared to that of dispersed structures, but differences fell within the standard deviations. No significant differences between changes in temperature could be found in the interface formation energy.

Potential Energy [kcal/mol]					
	E_{total}		$E_{lipid,single}$		E_{water}
	Dispersed	Island	DPPC	MPPC	
300 K	-65600 ± 182	-65700 ± 173	172 ± 8.53	120 ± 7.24	-69400 ± 152
305 K	-64800 ± 176	-64800 ± 184	169 ± 11.0	124 ± 8.36	-68800 ± 146

Interface Formation Energy [kcal/mol]		
	Dispersed	Island
300 K	-135 ± 8.14	-131 ± 10.5
305 K	-136 ± 8.22	-131 ± 10.4

Table 2: Interface Formation Energy

Radial distribution function

The radial distribution function (or RDF) is a form of a pair correlation function. It is used to demonstrate how atoms within a system are tightly packed together. This also is a good representation of average structure. RDFs were calculated to show how lipid heads of DPPC interacts with other DPPC and MPPC in the structure—and whether that interaction showed significant difference between dispersed and island structures. The results of island structures are in progress of simulation. Therefore, comparison between the two structures cannot be made yet. All of the graphs below are from dispersed strucrures.

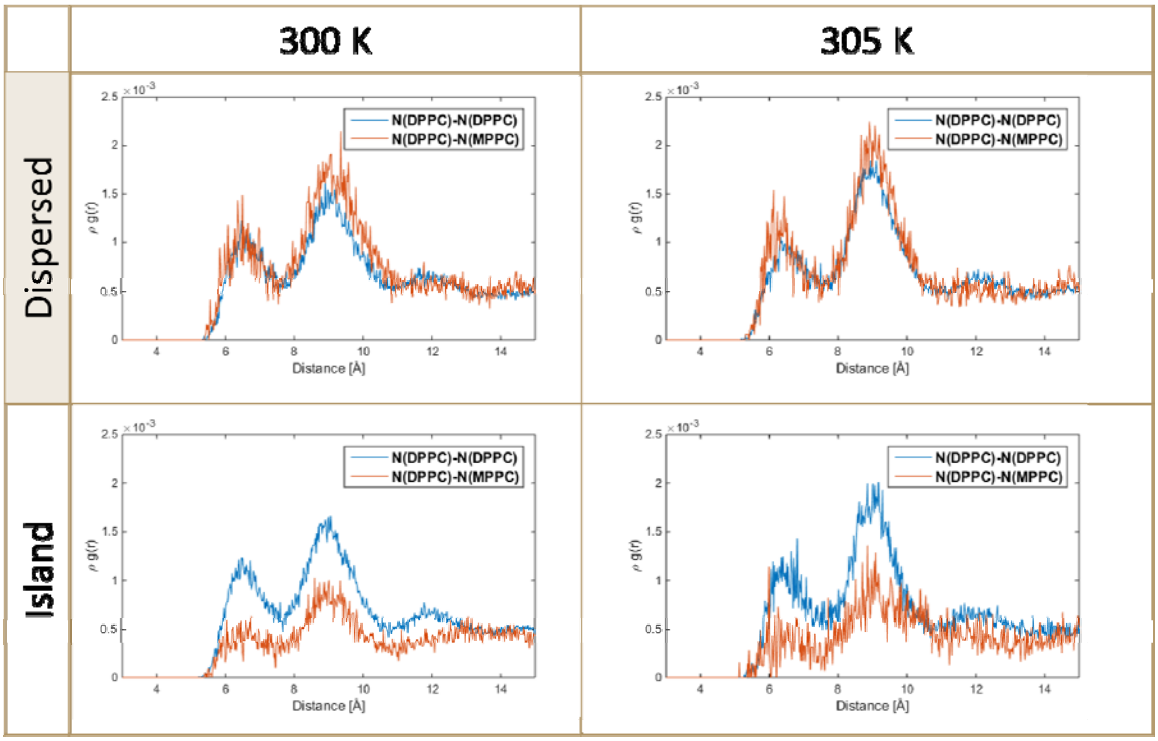

Figure 1: Radial distribution function on N(DPPC)-N(DPPC) and N(MPPC)-N(DPPC)

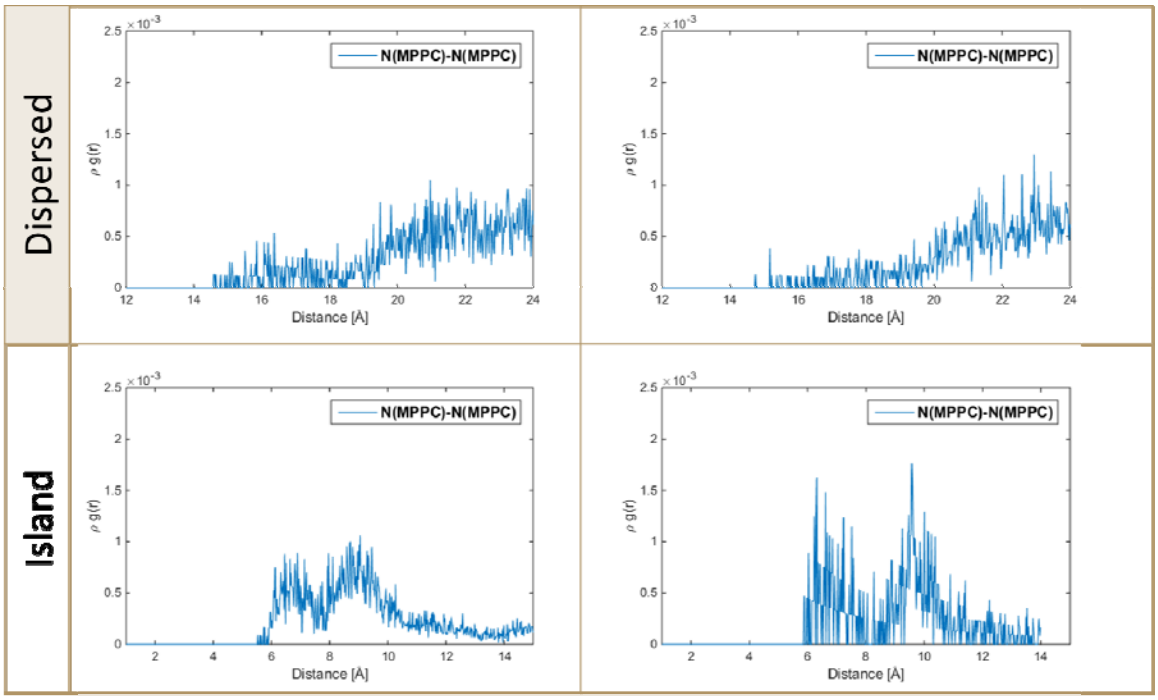

Figure 2: Radial distribution function on N(MPPC)-N(MPPC)

In dispersed structures, the comparison between interactions of DPPC to MPPC and DPPC to DPPC shows that there is no significant difference between the two (Figure 1). The comparison of MPPC to MPPC shows that most of them are at least 20 Å apart from each other (Figure 2). In island structure, we predict that the comparison between interactions of DPPC to DPPC to be similar to that of dispersed structure. However, for DPPC to MPPC structure in island structure to have a lower intensity as more of them are surrounded by MPPC rather than DPPC. As MPPC are aggregated together, we can expect to see MPPC to MPPC to have much shorter distance.

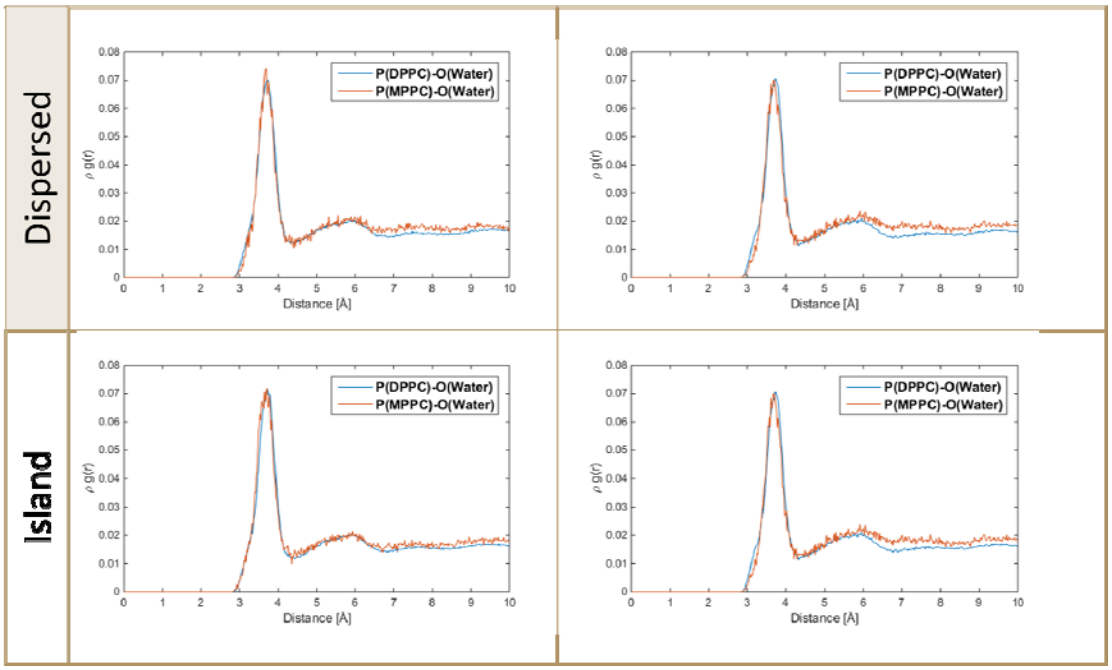

Figure 3: Radial Distribution Function on P(DPPC)-O(Water) and P(MPPC)-O(Water)

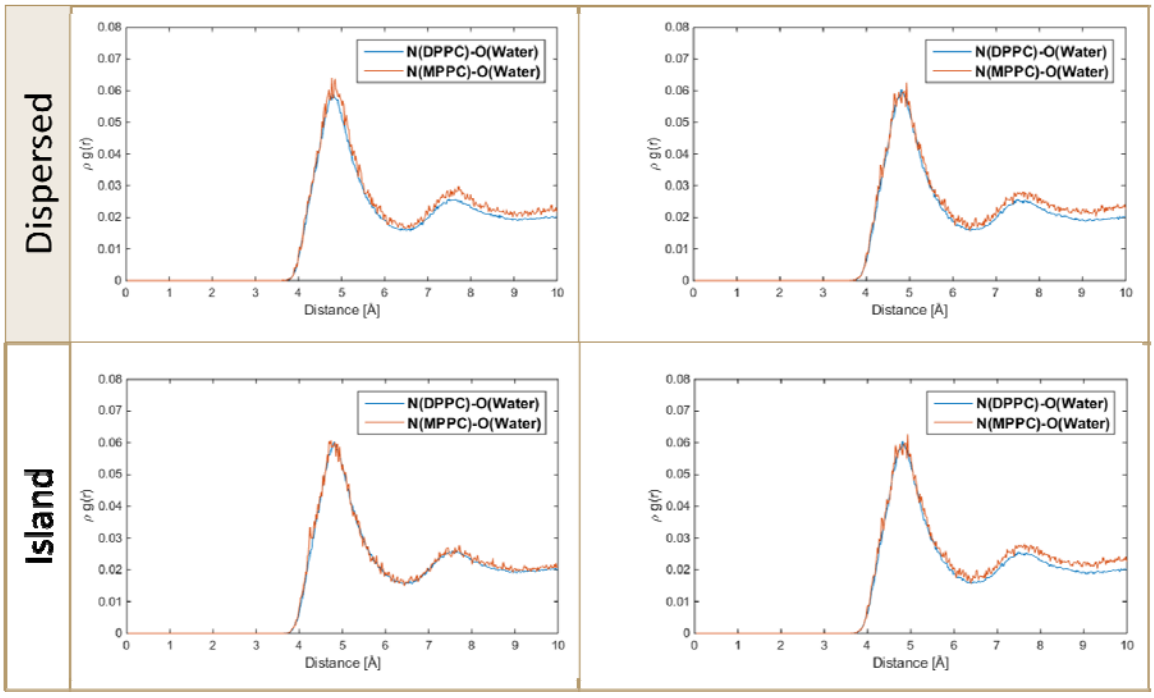

Figure 4: Radial Distribution Function N(DPPC)-O(Water) and N(MPPC)-O(Water)

Measuring the interaction of water with the head of lipid group, nitrogen have a longer distance to the water as their placement is closer to the lipid compared to that of phosphate. No significant differences between DPPC and MPPC to water has been observed, but MPPC showed slightly higher intensity than that of DPPC. Similar results are expected for Island Structures as well.

Density Profile

Density profile of dispersed structures is presented below (Figure 5). The density of water outside of lipid shows values that are very close to one as expected. It also shows that the water does not go much further into the lipid structure as predicted. A dip in DPPC density demonstrate space between the tails. Higher temperature and island structures showed wider 'dip' in the graph, compared to that of lower temperature and Dispersed Structure. The lipid thicknesses of the island structures are slightly thicker than that of dispersed structures. Although the composition of the system are identical in both dispersed and island structures, their composition showed some differences in their density.

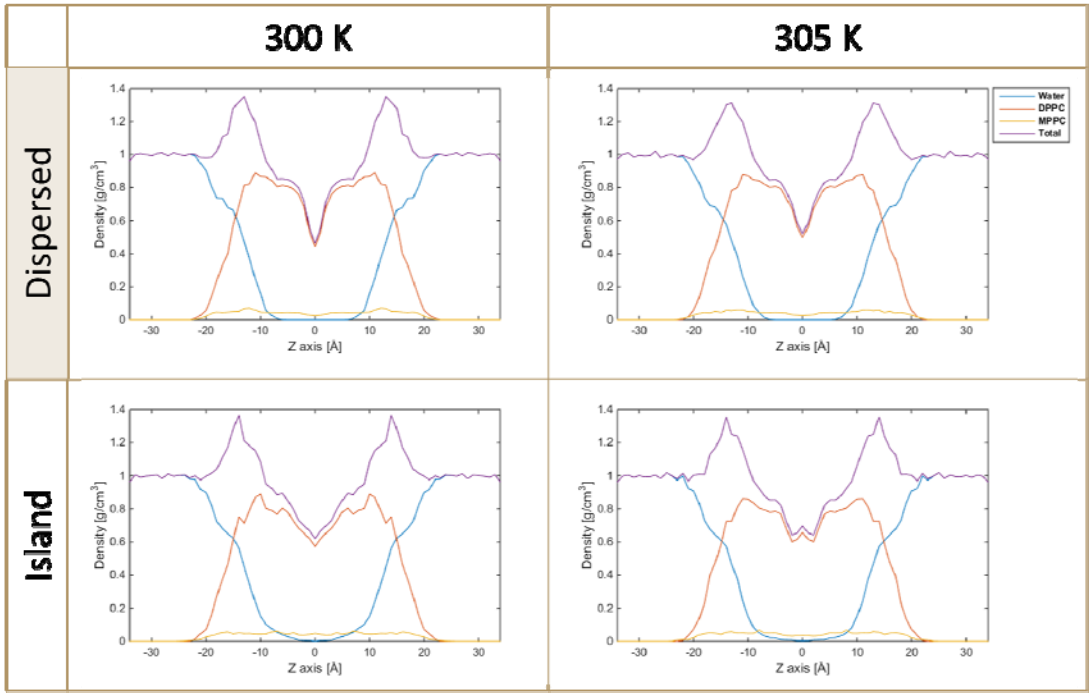

Figure 5: Density Profile

CHAPTER 5

CONCLUSIONS AND FURURE WORKS

Results and their analysis showed some differences in island and dispersed mixed lipid structures in gel phase. Further research on higher temperature, beyond transition temperatures at liquid crystalline phases, will be required to make any conclusive statements on difference between dispersed and island structures in mixed lipid bilayer structures. Also, there could be different results at different compositions of DPPC and MPPC structures. For example, if fifty percent of lipids are MPPC, rather than making up ten percent, effect of configuration may result in different results.

On the other hand, further analysis on the system will help understand more on potential similarities and differences between dispersed and island structures can be shown. For example, area per lipid might show if MPPC aggregated within MPPC behaves similarly to those aggregated within DPPC. It might also help explain different cell parameters of the simulation box that resulted from the simulation. Additionally, mean square displacement and further calculations on their diffusion coefficient will show if MPPC behaves similarly or differently in dispersed and island structures.

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